*ATP FORMATION CAUSED BY ACID-BASE TRANSITION OF SPINACH CHLOROPLASTS**

By André Tridon Jagendorf and Ernest Uribe†

McCollum-Pratt Institute and Biology Department,
The Johns Hopkins University, Baltimore

Communicated by W. D. McElroy, November 3, 1965

Previous work[1–5] has shown the existence of a high-energy condition of isolated chloroplasts, caused by illumination in the absence of phosphate or ADP. This state is inferred from the ability to form ATP in the postillumination darkness. Accompanying the condition is an apparent uptake of hydrogen ions[6, 7] together with an excretion of Mg^{++} or other cations.[8] A feasible interpretation, stemming from the "chemi-osmotic" hypothesis for the mechanism of phosphorylation in double membrane containing organelles,[9, 10] is that illumination causes the uptake of hydrogen ions into the inner space of the grana disk double membranes. The resulting inequality in hydrogen ion electrochemical activity across the membrane is postulated to be, in itself, the high-energy condition, able to drive the formation of ATP.

If this interpretation is correct, then the same high-energy condition should be formed artificially, entirely in the dark and without electron transport, by loading the inner space of the grana disk membranes with protons. If this could be accomplished by placing chloroplasts in an acid medium, when first returned to pH 8 they might be expected to make some ATP due to the pH gradient across the membranes. The operational formation of ATP in this manner, entirely in the dark, was discovered and noted briefly earlier.[4] The present paper represents an extension and further exploration of this phenomenon of "acid-bath" dark phosphorylation by chloroplasts.

Materials and Methods.—Chloroplasts were prepared from market spinach as described previously.[2] After the first centrifugation, they were resuspended in: 1.0 *M* sucrose, 0.01 *M* NaCl, and 5% dimethylsulfoxide, then frozen and stored under liquid nitrogen. (The use of dimethylsulfoxide has been found to aid in the preservation of photophosphorylation activity during freezing and thawing.) The chloroplasts were thawed before use and diluted to a concentration of 0.133 mg of chlorophyll per ml with: 0.80 *M* sucrose, 0.02 Tris pH 8.0 (at 0°C), 0.01 *M* NaCl, then centrifuged. They were then resuspended in 0.01 *M* NaCl alone, to the same concentration as in the previous step, and allowed to stand 20 min at 0°C for breakage. The broken chloroplasts were collected by centrifuging at 10,000 × *g* for 10 min and finally resuspended in 0.01 *M* NaCl at 0.50 mg of chlorophyll per ml. These broken and washed chloroplasts were used routinely, although occasional experiments gave identical results when suspension in sucrose-dimethylsulfoxide and freezing were omitted. All preparative steps were carried out at or close to 0°C.

The routine reaction procedure consisted of two successive stages, both carried out at 0°C: (*a*) exposure of chloroplasts to an acid pH, then (*b*) simultaneously raising the pH and adding ADP and phosphate to permit phosphorylation to occur. In the first stage chloroplasts containing 0.25 mg of chlorophyll were injected into 0.40 ml of buffer at pH 4.0 (or other as noted) containing 27 mμmoles of DCMU. The total volume at this point was 0.9 ml. After 60 sec, the acidified chloroplasts were taken up in a syringe and injected into a second test tube containing in 0.9 ml volume: Tris, 100 μmoles; ADP, 0.2 μmole; inorganic phosphate, 2.0 μmoles; $MgCl_2$, 5 μmoles; carrier-free radioactive phosphate with 5 × 10^5 counts per min, and enough NaOH to neutralize the buffer used in the acid stage. All reaction mixture components (exclusive of, and prior to addition of the NaOH) were at pH 8.0 or some other as indicated. Since the NaOH was neutralized by addition of the acid chloroplast mixture, the final pH was that of the buffer in the

second stage of the reaction. This final pH was checked directly in each experiment. The phosphorylation reaction (in final volume of 1.8 ml) was allowed to proceed for 15 sec, then stopped by the addition of 0.2 ml of 20% trichloroacetic acid. After centrifugation of denatured chloroplasts, the esterified radioactive phosphate was determined by the method of Avron,[11] or occasionally adsorbed to charcoal, washed, and counted.[2] Chlorophyll was determined by the method of Arnon.[12] In measuring ATP formation by the firefly luciferase assay,[13] three-times-recrystallized enzyme was used with reduced firefly luciferin as substrate.

Results.—In the first experiments on this phenomenon, great precautions were taken to exclude light; these included a 1–2-hr preincubation of the chloroplasts in complete darkness before acidification. Subsequent work showed, however, that adding the following inhibitors had absolutely no effect on the yields of ATP: 30 μM CMU, 10 or 30 μM DCMU, 50 μM o-phenanthroline, or 10 μM simazine. After this, DCMU was added routinely, at a final concentration of 3×10^{-5} *M*. Under these conditions (strong inhibitor of noncyclic electron transport, and no redox dye present), the chloroplasts were found to be completely inert to either room light or 5000 ft-c of white light; this was true even in the second stage with ADP and P^{32} present. Because of this, it has been possible to perform the experiments routinely in room light in an ice bath.

Formation of ATP due to making chloroplasts first acid, then alkaline, is shown in Table 1. There is substantial agreement between ATP formation as measured by luciferase, and by the incorporation of P^{32} followed by isobutanol extraction of the inorganic phosphate. The 20 per cent discrepancy between the two methods is under further investigation. It can be seen that ATP formation is absolutely dependent on achieving a pH below 7 in the acid stage, and on added phosphate and ADP in the alkaline stage. The 25 per cent yield in the absence of added Mg^{++} is presumably due to internal magnesium ions. Further identification of the product as ATP comes from the fact that all of the incorporated counts can be adsorbed on charcoal, even if the charcoal has been previously coated with stearic acid.[14] Once adsorbed, the counts are removed in boiling 1 *N* HCl at a rate identical to the hydrolysis of known ATP adsorbed under similar conditions.

The amount of ATP that is formed is highly dependent on the nature of the acid used to bring the chloroplasts down to pH 4. Figure 1 illustrates the yields obtained when using only HCl to adjust the pH, or when using various concentrations of glutamate, of succinate, or of phthalate buffers at pH 4. HCl in this experiment gave a yield of 17 mμmoles per mg chlorophyll, and in other experiments varied between 8 and 18. The same yield is obtained from 3 mM of glutamate. When checking the effects of other buffering anions, therefore, they were routinely added

TABLE 1

ATP FORMATION DUE TO ACID-BASE TRANSITION

Reaction mixture	Acid pH	Luciferase Assay		P-Molybdate Extraction		
		Total	Net*	Cpm	Total	Net
Complete	3.8	141†	129†	2200	166†	163†
"	7.0	12	—	45	3	—
$-PO_4$	3.8	12	—	—	—	—
−ADP	3.8	4	—	47	3	—
−Mg	3.8	60	48	630	48	45
−Chloroplasts	3.8	7	—	50	3	—

Complete, in this case, indicates an acid stage at pH 3.8 with 10 mM succinate and 3×10^{-5} *M* DCMU; and phosphorylation stage at pH 8.0 with components as indicated in *Methods* section.

* Net refers to the yield of ATP after subtracting the control of chloroplasts brought only to pH 7 before the phosphorylation stage.

† mμmoles ATP per mg chlorophyll.

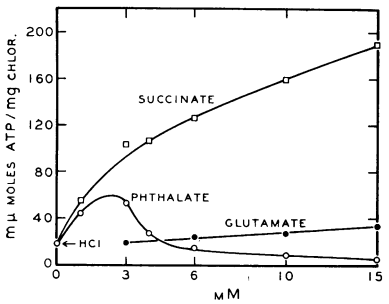

FIG. 1.—Yield of ATP as a function of acid concentration. Acid stage pH was 4.0, caused by addition of either 0.1 ml of 5 mM HCl, or by glutamate at the concentrations shown. With succinate or phthalate, 3 mM of glutamate was present in all reactions to achieve primary control of pH, and the other organic acid effects are over and above those due to pH 4 in itself.

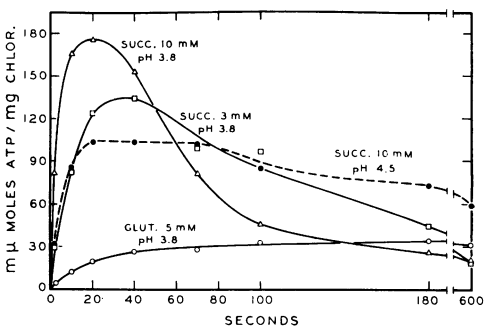

FIG. 2.—Time course for rise and decay of the energetic condition at pH 3.8 or 4.5. Glutamic or succinic acids used at the concentrations shown; conditions as in *Methods* section.

over a background of either 3 or 5 mM glutamate to control the pH. Phthalate, the first additional buffer tried, increases the yield from 17 up to 60 mμmoles per mg chlorophyll, but an excess is inhibitory. Inhibition depends on the pH; the same concentrations at pH 4.5 do not inhibit, and the concentration curve is less sharp. Succinate provides higher yields of ATP, with no sign of inhibition up to 15 mM. The largest amount of ATP formed in the experiment of Figure 1 was 189 mμmoles per mg chlorophyll; in other experiments yields up to 240 (i.e., 43 μmoles per gram of protein) have been seen routinely, and higher yields very occasionally.

Time courses for the rise of the energetic conditions in acid are shown in Figure 2. Glutamate gives a rise to 85 per cent of the maximum in about 40 sec, then a very slow increase to slightly higher yields by 3 min. Succinate addition produces a time course which reaches a maximum by 20 sec. With succinate, the half time for the rise is 8 sec for 3 mM and 2 sec for 10 mM at pH 3.8, and 4 sec for 10 mM at pH 4.5. Thus the effect of higher succinate or the lower pH is not to prolong the time course, but to give a faster initial rate and a higher plateau. The succinate curves in this experiment show unusual instability; in most experiments the high-energy condition was stable for at least 60 sec at pH 3.8. It is clear that any instability of the high-energy potential is greater as the pH drops below 4.5.

Time courses at pH 8 are shown in Table 2. By omitting the ADP and P^{32} for varying lengths of time the rate of decay can be estimated; at pH 8 the decay reaction appears to be complete in about 6 sec and seems to have a half life of between 1 and 2 sec. Phosphorylation of ADP is complete in about 4 sec.

Besides succinate, the following acids have shown good activity at 1–3 mM: o-phthalic, p-phthalic, barbituric, fumaric, dimethylglutaric, lactic, and glycolic. Dicarboxylic acids with hydroxyl groups have been either weakly active or inhibitory, and all monofunctional carboxylic acids tested have been strongly inhibitory. Further studies of the structural requirements for induction of ATP formation, or for inhibition, are currently in progress.

Reversibility of acid induction is shown in Table 3. It is possible for one batch

TABLE 2

KINETICS OF DECAY OF ACID-INDUCED X_E AT pH 8, AND OF PHOSPHORYLATION IN THE ADP STAGE

Reaction	Yield of ATP at Time (in sec)					
	0	2	4	6	30	60
pH 8 Decay	41	7	1.7	1.1	0.4	—
ADP + P → ATP	3	27	48	47	42	47

ATP yield in mμmoles/mg chlorophyll. The acid stage contained 10 mM phthalate at pH 4.6. The alkaline decay was measured by injecting the acid chloroplasts into Tris at pH 8, then waiting the indicated number of seconds before adding ADP, Mg, and P^{32}, followed by a standard 15 sec with these reagents before adding TCA. The phosphorylation time course was determined by injecting acidified chloroplasts into the complete phosphorylation stage reaction mixture, and waiting the indicated number of seconds before stopping the reaction with TCA.

TABLE 3

REVERSIBILITY OF INDUCTION OF X_E BY ACID

Acid	pH in Successive Minutes			ATP
	1	2	3	
Glutamic (5 mM)	4.0	—	—	15.6*
	4.0	6.5	—	0.0
	4.0	6.5	4.0	13.7
Succinic (5 mM)	4.0	—	—	49.7
	4.0	6.5	—	2.8
	4.0	6.5	4.0	33.6

* mμmoles ATP per mg chlorophyll. The pH, initially 4.0, was adjusted up to 6.5 by adding a measured amount of Tris as the pure base, then readjusted back to 4.0 by adding a measured amount of the free acid. The phosphorylation stage components were added only after the last of the pH transitions noted in the first three columns; thus, the discharge at pH 6.5 represents instability of X_E near neutral pH. ATP formation occurred on addition of the usual phosphorylation stage ingredients at pH 8 for 15 sec, except that in this particular experiment the succinic and glutamic acids were not neutralized by extra NaOH.

of chloroplasts to become charged at pH 4, discharged at pH 6.5, and then almost fully recharged when returned to pH 4 for the second time. This whole sequence is accomplished before addition of ADP and phosphate.

Table 4 shows that ATP formation in this system can be inhibited by the known uncouplers of photosynthetic phosphorylation. Ammonia, atebrin, CCP, Triton-X-100, phenylmercuric acetate, and uncoupling by EDTA are all reasonably effective. On the other hand, a better yield was found anaerobically than aerobically (160 vs. 109 mμmoles ATP per mg chlorophyll).

The yield of ATP as a function of pH in the acid stage is shown in Figure 3, for the case of glutamic acid. Very similar curves, although with much higher yields, are obtained when using succinic acid. Note that the yield goes up as the pH drops. No distinct pH optimum has been observed because at pH 3.5 the chloro-

TABLE 4

INHIBITION OF ATP FORMATION BY UNCOUPLERS OF PHOTOPHOSPHORYLATION

Inhibitor	Yield	% Inhibition
None	239*	—
NH_4Cl, 2 mM	61	75
Atebrin, 0.4 mM	57	76
PMA, 50 μM	59	75
CCP, 10 μM	135	43
Triton, 0.27 mg	110	54
EDTA	18	92

* mμmoles ATP per mg chlorophyll. Acid stage contained 10 mM succinate at pH 4.0, ADP stage pH was 8.3. PMA refers to phenylmercuric acetate, CCP to carbonyl cyanide m-chlorophenylhydrazone, and EDTA to ethylenediamine tetraacetic acid. The concentrations are those in the acid stage; in the phosphorylation stage they would be twice as dilute. Chloroplasts were preincubated for 5 min at 0°C with the CCP and PMA; in the case of EDTA they were resuspended in 1 mM EDTA in place of the 10 mM NaCl during their preparation, and washed free of the EDTA and coupling factor before use.

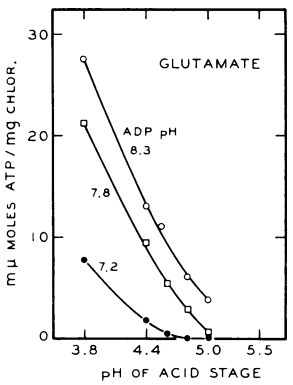

FIG. 3.—Yield of ATP as a function of acid stage pH. Five mM glutamate was used in the acid stage. Similar curves, although with higher yields, were found when using succinate, between 1 and 10 mM.

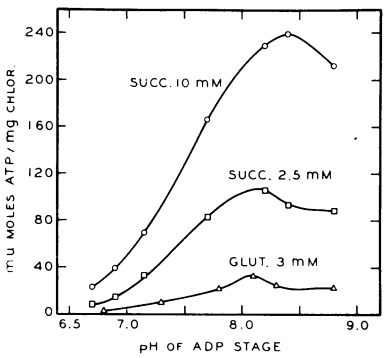

FIG. 4.—Phosphorylation stage pH curves, with differing amounts of succinate (pH 4.0 in each case) in the acid stage. Each amount and pH of succinate was neutralized by an appropriate amount of NaOH, and the pH of the phosphorylation mixture was checked at 0°C.

plasts aggregate severely and it is no longer possible to use the usual syringe procedures. Both the cutoff point on the alkaline side and the total yields are raised when the pH of the phosphorylation stage is higher.

Phosphorylation stage pH curves are shown in Figures 4 and 5. There is a distinct optimum, but its position shifts depending on conditions in the acid stage. With decreasing yields due to lowering the succinate concentration from 10 to 2.5 mM (Fig. 4), the optimum phosphorylation stage pH goes *down*, from 8.4 to 8.1. By contrast, if the yield is reduced by using an insufficiently acid pH in the first stage (Fig. 5), the pH optimum of the phosphorylation stage *rises* from 8.1 to 8.6 (glutamate, *lower curves*) or from 8.4 to 9.0 (succinate, *upper curves*). Not only the optimum, but also the cutoff point on the acid side, is forced up about 0.6 pH units as the acid stage pH is raised from 3.8 to 4.8. In short, formation of ATP seems to depend not only on the pH in each stage, but also to a large extent on the actual size of the *difference* in pH between the two stages. The minimal pH difference for synthesis of measurable ATP ranges from 2.2 to 2.9 units, and the optimum from 4.0 to 4.5 units.

Discussion.—The experiments reported here show that ATP is formed by chloroplasts depending only on an artificial transition from acidic to basic medium. This ATP synthesis occurs certainly without the aid of light, and quite possibly without any electron transport. Nevertheless, it seems that a large part of the ordinary photophosphorylation machinery must be in operation, as shown by sensitivity to a representative group of uncouplers (Table 4). Especially significant is severe inhibition by EDTA treatment, known to remove the chloroplast "coupling factor."[15] The uncoupler effects, the kinetics of the pH 8 decay reaction, and of the phosphorylation proper (Table 2) are virtually identical to those seen previously for the high-energy conditions, X_E, induced by light.[2, 16] Light-induced X_E in turn had been closely related to a light-induced rise in pH of the medium, indicating acidification of the interior of the chloroplasts.[6, 7]

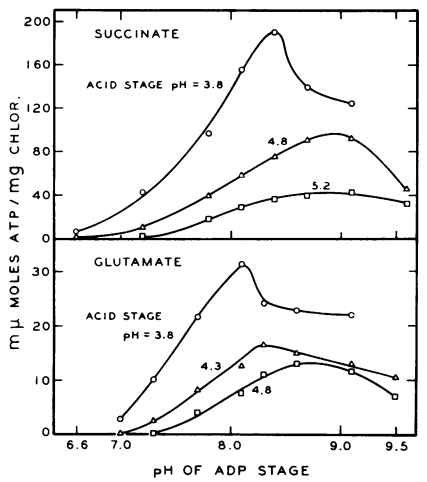

FIG. 5.—Phosphorylation stage pH curves, with either succinate (*top set of curves*) or glutamate (*bottom set*) in the acid stage, at different acid stage pH's as indicated. The acids were neutralized with NaOH in the ADP mix in all cases, and the final pH was checked at 0°C.

No effort has yet been made to find the absolute maximal yield of ATP. Occasional experiments have shown values higher than 240 mμmoles per mg of chlorophyll and, as noted with respect to the light X_E,[5] the potential yield might be twice as much as the observed one. In any event it is clear that the current procedures lead to formation of at least 1 ATP per 4 chlorophylls, or 100 ATP molecules per cytochrome *f*.

A new factor in the present work is the important function of added organic acids, especially dicarboxylic acids. The basal level, caused by pH 4 in itself or by glutamate, is increased about 15-fold by including succinate at 10 mM. The function of these acids is far from clear; however, the lack of specificity (e.g., o- and p-phthalic acids, succinate, barbiturate, glycolate) makes it unlikely that they are serving as substrates for an enzymatic reaction.

The thermodynamic basis for formation of ATP in these experiments is not known. The data we have obtained can be used to assess the likelihood of a number of alternative, speculative explanations:

(*a*) At the lower pH a covalent anhydride bond involving chloroplast membrane components might conceivably form spontaneously and reversibly (as, for instance, in the equilibrium between succinic acid and succinic anhydride). On returning to pH 8.3 this could be at a high energy level, and by a series of group transfers lead to ADP phosphorylation. Decrease of the free energy level of pyrophosphate and thioester bonds with decreasing pH was noted considerably earlier.[17]

(*b*) There might exist two electron carriers whose redox potential relationship reverses itself on going from pH 3.8 to 8.3. ATP formation in this case would be coupled to anaerobic electron transport at the higher pH:

$$\text{at pH 3.8:} \quad A + BH_2 \rightarrow B + AH_2$$

$$\text{at pH 8.3:} \quad B + AH_2 + ADP + P \rightarrow A + BH_2 + ATP$$

However, three of our observations tend to rule out both of these possibilities, at least in their simplest form. These are: (1) the very high yields of ATP, higher than that of any known bound electron transport component. Even plastoquinone A is present at the level of only 40 moles/cytochrome *f*; and all of the fatty quinones together come to probably 80 moles/cytochrome *f*. As noted above, ATP yields are certainly 100 moles/cytochrome *f*, and may be much higher. (2) The important effect on yield of a rather nonspecific group of added organic acids is entirely inconsistent with possibility (*b*) above, and consistent with (*a*) only if the organic acid added were to participate in formation of an anhydride bond. (3) The shift in the pH curve corresponding to different acid stage pH's (Fig. 5) is not readily consistent with any model in which a static amount of a chemical or redox component is formed

in the acid stage, and conversion to ATP depends only on the pH optima for the chemical reactions in the last stages of phosphorylation. Note especially that it is the most abnormal pH (3.8) which produces a phosphorylation reaction pH optimum (8.1 or 8.3) closest to that seen normally for photophosphorylation.

On the other hand, these facts are, on the whole, consistent with (*c*) the "chemiosmotic" hypothesis for the mechanism of phosphorylation, as elaborated by Peter Mitchell.[9, 10] In this model, account is taken of the inner and outer regions of the grana disk membranes. When placed in acid, protons would be expected to penetrate the interior space depending on the external pH. Presumably, cations would be simultaneously displaced to preserve electrical neutrality. Added organic acids could penetrate in the undissociated form (without the need to expel cations) and provide an internal reservoir of dissociable protons, thereby accounting for the higher yields. On raising the external pH to 8 or more, a proton gradient would be present from inside to outside, and this in itself would have thermodynamic potential. The height of the potential would depend on the ratio of pH's inside and outside, and this could account for the requirement of a distinct pH difference between the acid stage and the phosphorylation reaction. Indeed, the alkaline displacement of the phosphorylation reaction pH curve due to raising the acid stage pH (Fig. 5) was one of the predictions of this model, and the experiments were performed in order to test it. The actual yield of ATP at any pH, and therefore the shape of the curve, probably reflects a balance between the pH dependence of the relevant chemical reactions, and the thermodynamic effect which is a matter of the change in pH from the previous acid stage.

Given a pH differential across the grana disk membrane, its potential might be translated into ATP formation via an anisotropic, membrane-bound, reversible ATPase as discussed by Mitchell.[9, 10] Alternatively, one might imagine a pair of membrane-bound, phosphorylation-coupled electron carriers with potential sensitive to pH (see above) able to "face" alternatively the acidic inside and alkaline outside regions. By continuous movement from one side to the other, a continuing coupled electron transport might be achieved, with a final yield of ATP higher than the net amount of either carrier. Although we consider this a less likely alternative, none of our present evidence can rule it out.

Summary.—Chloroplasts form a limited amount of ATP without illumination or oxygen if made first acid, then basic. The yields are greatly increased by having an appropriate organic acid present in the acid stage. Lack of specificity of the acid suggests it does not serve as a substrate for a specific enzyme. The highest yields obtained regularly have been 1 ATP for every 4 chlorophylls (i.e., 100 ATP per cytochrome *f*, or 40 μmoles ATP per gram of protein). Formation of ATP is sensitive to known uncouplers of photosynthetic phosphorylation, and the kinetics of either decay of the intermediate or of phosphorylation at pH 8 are the same as those for the high-energy condition induced by illumination at pH 6. The yield of ATP depends in part on the actual pH differential between the two experimental stages. The data are suggested to be consistent with a model in which the high-energy condition consists of a pH gradient across the grana disk membranes.

The devoted assistance of Marie Smith is gratefully acknowledged, as well as help from Miss Rosemary Kayser with the luciferase assays.

Abbreviations used in this paper include CMU for p-chlorophenyl-1, 1-dimethylurea; DCMU for dichlorophenyl-1,1-dimethylurea.

* Publication 455 of the McCollum-Pratt Institute. This work was supported in part by grant GM-03923 from the National Institutes of Health, and by a Kettering Research Award.

† National Science Foundation postdoctoral fellow.

[1] Shen, Y. K., and G. M. Shen, *Sci. Sinica (Peking)*, **11**, 1097 (1962).

[2] Hind, G., and A. T. Jagendorf, these PROCEEDINGS, **49**, 715–722 (1963).

[3] Hind, G., and A. T. Jagendorf, *Z. Naturforsch.*, **18b**, 689–694 (1963).

[4] Hind, G., and A. T. Jagendorf, *J. Biol. Chem.*, **240**, 3195–3201 (1965).

[5] Jagendorf, A. T., and G. Hind, *Biochem. Biophys. Res. Commun.*, **18**, 702–709 (1965).

[6] Neumann, J., and A. T. Jagendorf, *Arch. Biochem. Biophys.*, **107**, 109–119 (1964).

[7] Jagendorf, A. T., and J. Neumann, *J. Biol. Chem.*, **240**, 3210–3214 (1965).

[8] Dilley, R. A., and L. P. Vernon, *Arch. Biochem. Biophys.*, **111**, 365–375 (1965).

[9] Mitchell, P., *Nature*, **191**, 144–148 (1964).

[10] Mitchell, P., in *Regulation of Metabolic Processes in Mitochondria*, ed. E. Quaglieriello, E. C. Slater, S. Papa, and J. M. Tager (Amsterdam: Elsevier Press, 1966), in press.

[11] Avron, M., *Biochim. Biophys. Acta*, **40**, 257–271 (1960).

[12] Arnon, D. I., *Plant Physiol.*, **24**, 1–15 (1949).

[13] McElroy, W. D., in *Methods in Enzymology*, ed. S. P. Colowick and N. Kaplan (New York: Academic Press, 1955), vol. 2, pp. 851–856.

[14] Alm, R. S., *Acta Chem. Scand.*, **6**, 1186–1193 (1952).

[15] Avron, M., *Biochim. Biophys. Acta*, **77**, 699–702 (1963).

[16] Hind, G., and A. T. Jagendorf, *J. Biol. Chem.*, **240**, 3202–3209 (1965).

[17] Burton, K., and H. A. Krebs, *Biochem. J.*, **54**, 94–105 (1953).

---

*ON THE RELATIONSHIP BETWEEN VITAMIN D ACTION AND ACTINOMYCIN-SENSITIVE PROCESSES**

BY J. ELWOOD ZULL, E. CZARNOWSKA-MISZTAL, AND H. F. DELUCA

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN, MADISON

Communicated by David E. Green, October 26, 1965

Vitamin D apparently prevents rickets by maintaining the serum $(Ca^{++})\cdot(HPO_4^{=})$ product at a level which is supersaturated with respect to bone mineral.[1] The vitamin elevates serum calcium and phosphate by causing increased absorption of these ions from the intestine,[2] and mobilizing mineral from formed bone.[3] The former phenomenon, at least, is due to stimulated active transport of calcium[4, 5] and, secondarily, phosphate.[6]

The nature of the action of vitamin D in stimulating calcium transport is not known. However, the demonstration that actinomycin D blocks the physiological action of vitamin D[7–10] led us to suggest that the vitamin induces the synthesis of a protein component of a calcium transport system. The present paper extends this observation and presents a modified version of our earlier hypothesis concerning vitamin D action.

Materials and Methods.—All of the methods used in this paper have been described elsewhere.[7, 11–13] Ca^{45} and P^{32} were obtained from Oak Ridge National Laboratories, Oak Ridge, Tenn. Actinomycin D was a gift from Merck, Sharp and Dohme Research Laboratories, and the Cancer Chemotherapy Center, National Cancer Institute, Bethesda, Md. Puromycin was a gift from Dr. Joyce Mohberg,